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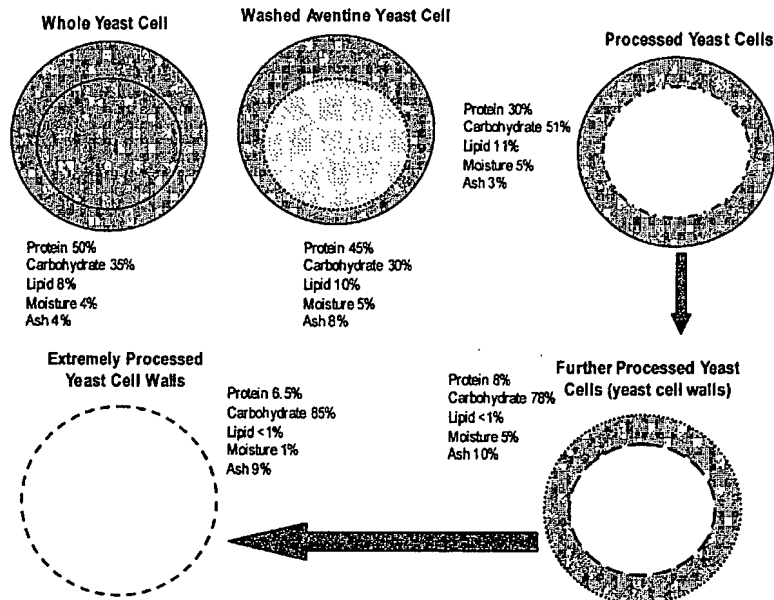
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(54) Title: COMPOSITION

#### Biochemical Differences Between Whole Yeast and Yeast Cell Walls



(57) Abstract: A composition comprising a microcapsule and an encapsulatable material, wherein the microcapsule comprises an autolysed microbial cell having a cell wall, wherein the encapsulatable material is substantially encapsulated within the cell wall and a method of encapsulation.

## COMPOSITION

The present invention relates to a composition and a method of encapsulation.

The use of microorganisms for encapsulating materials for protecting and/or delivering active ingredients has been widely disclosed in the prior art. A method of producing microbial encapsulated materials is proposed in US 4001480.

According to that Specification, microbes typified by fungi are cultivated to produce a very high natural fat content, i.e. microbial lipid content, of about 40 to 60% by weight, and the microbes are placed in contact with materials which are soluble in the microbial lipid so that the materials pass into the lipid and are retained passively therein.

Another method of producing microbial encapsulated materials is described in EP 0085805. In that method, microbes typified by fungi which may have a microbial lipid content significantly less than 40% by weight are treated with defined organic liquid lipid-extending substances and with materials which are soluble or micro-dispersible in those substances so that both the lipid-extending substance and the material which is soluble or micro-dispersible therein enter and are retained passively within the microbe.

The aforementioned prior methods rely either on special microbe cultivation conditions to enhance the microbial lipid content to a very high level or on the use of a lipid-extending substance, and the materials to be encapsulated must be

either soluble in the microbial lipid or soluble or micro-dispersible in the lipid-extending substance, respectively.

FR 2179528 describes a method of causing certain materials to be absorbed and/or fixed by microbes, in which a microbe is treated with a plasmolyser, i.e. a substance which causes contraction or shrinking of the microbial cytoplasm by exosmosis of cytoplasmic fluid, and then an aqueous solution of neodymium chloride, magnesium chloride or onion juice is added under certain conditions so that the aqueous material is absorbed in place of the extracted cytoplasmic fluid.

EP0242135 describes a process whereby grown, alive and intact yeast are grown and then used immediately for encapsulation purposes. Such yeast cells typically have a high fat content.

However, none of the prior art methods provide a final product having sufficient loading of exogenous material nor do they provide a sufficiently efficient method so as to give rise to a commercially viable process for producing microbially encapsulated actives etc.

It is an object of the present invention to overcome or alleviate one or more of the problems associated with the prior art.

In accordance with a first aspect of the present invention, there is provided a composition comprising a microcapsule and an encapsulatable material, wherein the microcapsule comprises an autolysed microbial cell having a cell wall, wherein the encapsulatable material is substantially encapsulated within the cell wall.

Surprisingly, the applicants have discovered that it is possible to encapsulate an active inside yeast cell walls with damaged cell membranes or yeast cell walls such as ghost cells. The present invention describes the use of microorganisms, particularly yeast cells, which have been autolysed for example during the yeast extract production process. In this case the microorganism, particularly yeast, cell wall is intact but the cell membrane is damaged, partially, or completely or not present at all.

The microcapsule may be derived from a fungal cell, bacterial cell or algae cell. Preferably, the microcapsule is derived from a fungal cell.

The microcapsule may be derived from one or more fungi from the group comprising *Mastigomycotina*, *Zygomycotina*, *Ascomycotina*, *Basidiomycotina* and *Deuteromycotina*. Preferably, the microcapsule is derived from one or more fungi from *Ascomycotina*. More preferably, the microcapsule is derived from yeasts. More preferably still, the microcapsule is derived from one or more of the group comprising *Candida albicans*, *Blastomyces dermatitidis*, *Coccidioides immitis*,

*Paracoccidioides brasiliensis*, *Penicillium marneffe*, *Saccharomyces bouladii* and *Saccharomyces cerevisiae*. Even more preferably still, the microcapsule is derived from *Saccharomyces cerevisiae*, such as common bakers yeast and yeast obtainable as a byproduct of ethanol biofuel production.

In contrast, the prior art discloses fungal cell in grown form for use as microcapsules, i.e. It has been harvested from its culture medium, and is intact, ie. not lysed. The fungal cell from which the microcapsules of the present invention can be derived may be alive, may be a ghost cell or may be dead, i.e. unable to propagate.

In one composition according to the present invention, the microcapsule has an average diameter of approximately 5 microns. The lipid content may be less than 60%, preferably less than 40%, more preferably less than 25%, still more preferably less than 15%, even more preferably less than 5%, and can still work at less than 1% by dry weight of the microcapsule.

In one embodiment the microcapsule is a by-product of a biofuel process i.e. the microcapsule is preferably derived from a biofuel yeast.

The composition may comprise a plurality of microcapsules. In one embodiment, the composition comprises a plurality of microcapsules derived from a plurality of different types of fungal cells.

Cells suitable for use in the present invention may be the byproduct of the yeast extract process or by processes where nucleotides, proteins and any other biochemical component / cell component are removed specifically from the yeast and the cell membrane may be intact or damaged. Preferably cells will have intact cell walls and may be described as cell walls

In addition because the yeast cell wall material can be used to entrap water soluble components then where active ingredients are mixtures of water soluble and water in-soluble components the encapsulated product is more authentic, for example a complex flavour (Strawberry).

Encapsulated compounds are described in WO 00/69440.

The cell wall preferably comprises at least 70% polysaccharides, more preferably at least 80% polysaccharides and most preferably at least 90% polysaccharides.

The polysaccharides may comprise any one or more of glucans, mannans and chitin.

The glucans present in the cell wall may comprise  $\beta$ -2,6 and  $\beta$ -1,3-linked glucans.

The mannans present in the cell wall may comprise a -1,6-linked inner core with a -1,2- and a -1,3 side chains.

The weight percentage of chitin in the cell wall preferably comprises at least 1%. More preferably, the percentage of chitin by weight in the cell wall is in the range 1-5%. More preferably still, the percentage of chitin by weight is in the range 2-4%.

The cell wall is preferably at least 70 nm thick. More preferably the cell wall is 100-200 nm thick

The protein content of the cell wall may be less than 50%, preferably less than 45%, more preferably less than 40%, still more preferably less than 30%, even more preferably less than 20%, and most preferably less than 10% by dry weight of the microcapsule.

The microbial cell may comprise at least 30 % by dry weight carbohydrate. Preferably, the cell comprises at least 50 %, more preferably 75% and even more preferably 85% by dry weight of carbohydrate.

The encapsulatable material may comprise any one or more of a flavour, a fragrance, a pharmaceutically active compound, a phytoactive compound, a biocidally active compound, an antimicrobial or microbialstatic compound, a

pesticidal compound, an insecticide, an avicide, an acaricide, a rodenticide, a molluscicide, a nematocide, a nutraceutical, an animal/bird/insect repellent and or attractant compound, a cleaning agent, adhesive or adhesive component, a dye, an antioxidant, a skin-anti-wrinkle agent, an essential oil or a pheromone.

The encapsulatable material may be lipophilic or may comprise a lipophilic moiety. Preferably, the active compound is lipophilic or substantially lipophilic.

The term 'substantially lipophilic' as used herein is meant to include those compounds having lipophilic and lipophobic moieties wherein the lipophilic moiety is predominant.

The encapsulatable material may be lipid soluble.

The encapsulatable material may be derived from a lipophobic compound and which is made lipophilic by chemical modification, such as for example esterification, the addition of an alkyl group etc. without substantially compromising efficacy of the encapsulatable material. Hydrophilic compounds may be rendered lipophilic by pH adjustment thus improving their suitability for encapsulation.

Alternatively, the encapsulatable material may be hydrophilic, hydrophobic or may comprise hydrophilic and hydrophobic moieties. Preferably, the pharmaceutically active compound is hydrophilic or substantially hydrophilic. The



term 'substantially hydrophilic' as used herein is meant to include those compounds having hydrophilic and hydrophobic moieties wherein the hydrophilic moiety is predominant. The pharmaceutically active compound is preferably water soluble.

The encapsulatable material may further comprise a carrier. For example, in one embodiment, the encapsulatable material is a crystalline solid dissolved in an organic solvent carrier. Thus, the carrier facilitates encapsulation of the encapsulatable material

The encapsulatable material may be derived from a lipophobic compound and which is made lipophilic by chemical modification, such as for example esterification, the addition of an alkyl group etc. without substantially compromising efficacy of the encapsulatable material, or by pH adjustment.

The encapsulatable material may further comprise a carrier. For example, in one embodiment, the encapsulatable material is a crystalline solid soluble in the presence of the carrier. Thus, the carrier facilitates encapsulation of the encapsulatable material.

The encapsulatable material is preferably in liquid form or solution. This is to facilitate encapsulation within the adjuvant. The encapsulatable material may be liquid in its normal state or it may be a solid, in which case it is preferably

dissolved or micro-dispersed in a carrier such as a solvent which is lipid soluble.

Suitable carriers include any one or more of the following:

- a) primary alcohols within the range C4 to C12, such as nonanol and decanol;
- b) secondary and tertiary alcohols;
- c) glycols, such as diethylene glycol;
- d) esters, particularly esters having straight carbon chains greater than 2 and less than or equal to 12, for example, ethyl butyrate, triacetin;
- e) aromatic hydrocarbons such as xylene and acetopenone;
- f) any aromatic lipophilic oil with no straight chain branch greater than 12 Carbons; and
- g) carboxylic acids between C3 and C12

The carrier is preferably non-miscible with water. Preferably, the carrier is organic and has a molecular weight in the range of 100 – 700. More preferably, the carrier is not miscible with water.

In one embodiment, the carrier comprises a mixture of 2 or more solvents. Preferably, at least one of the solvents is not miscible with water. More preferably, the mixture of solvents forms a homogeneous liquid mixture.

The carrier may comprise any one or more selected from the following: Alkanes, alkenes, alkynes, aldehydes, ketones, monocyclics, polycyclics, heterocyclics,

monoterpenes, furans, pyroles, pyrazines, azoles, carboxylic acids, benzenes, alkyl halides, alcohols, ethers, epoxides, esters, fatty acids, essential oils.

Preferably, the carrier is selected for a particular encapsulatable material. For example, phytotoxic carriers are less appropriate to herbicide applications.

By way of example the carrier may comprise any one or more of the following:

**Table 1 – carriers**

Name	logP(o/w)
1-(2-aminophenyl)-1-ethanone	1.1
Acetophenone (1-phenyl-Ethanone)	1.7
alpha pinene	3.9
alpha terpineol	1.7
Benzene	2.0
Benzonitrile	1.5
Benzyl alcohol	1.1
Bromobenzene	2.9
1-butanethiol	2.1
Butylbenzene	3.9
Caryophyllene	6.0
Chlorobenzene	2.6
Cyclohexane	3.2
Cyclohexanol	1.6
Decane	5.3
decanoic acid	3.5
5-decanolide	3.1
Decyl alcohol	3.8
diallyl disulfide	3.1
1,3-Difluorobenzene	2.4
Dimethyl adipate	1.4
3,4-dimethyl phenol	2.2
3,7-dimethyl-2,6-octadienal	1.7
1,5-dimethyl-1-vinyl-4-hexenyl acetate	2.7
1,5-dimethyl-1-vinyl-4-hexenyl hexanoate	4.5
dipropyl disulfide	3.7
(+)-5-dodecanolide	4.0
dodecanoic acid	4.4

Epibromohydrin	2.1
Ethylbenzene	3.0
ethyl (E)-3-hexenoate	1.7
4-ethyl-2-methoxy phenol	2.4
ethyl 3-methylbutanoate	1.8
ethyl hexanoate	2.3
ethyl nonanoate	3.7
Fluorobenzene	2.2
Heptane	3.8
1-Heptanol	3.1
heptan-2-one	1.9
Hexane	3.3
1-Hexanol	2.7
(Z)-3-hexenyl 2-methylbutanoate	2.8
(Z)-3-hexenyl acetate	1.5
(Z)-3-hexenyl butanoate	2.4
2-hydroxy benzaldehyde	1.5
Indole	2.3
Iodobenzene	3.2
3-Iodotoluene	3.7
isobutyl phenylacetate	3.2
4-isopropyl benzaldehyde	3.0
1-isopropyl-4-methylbenzene	4.0
5-isopropyl-2-methylphenol	3.1
2-isopropyl phenol	2.7
Limonene (1-methyl-4-(1-methylethenyl)-	
Cyclohexene	4.8
(+)-(S)-1(6),8-P-menthadien-2-one	1.0
(1R,4R)-8-mercapto-3-P-menthanone	2.9
Methyl benzoate	1.8
3-methyl butylamine	1.1
6-methyl quinolene	2.6
6-methyl-5-hepten-2-one	1.0
6-methyl-5-hepten-2-one	1.0
2-methyl hexanoic acid	2.1
s-methyl 3-methylbutanethioate	2.1
nonanoic acid	3.5
Nonane	4.8
1-Nonanol	3.3
(Z)-6-nonen-1-ol	2.3
octan-2-one	2.3
Octanol	2.8
1-octen-3-ol	2.7
octyl acetate	3.3
octyl isobutyrate	4.2

oleic acid	7.4
1-octyl-2-pyrrolidinone	3.3
Pentafluorobenzene	3.0
2-phenyl ethyl octanoate	4.7
2-phenylethyl 3-methyl-2-butenate	2.7
3-phenyl propanoic acid	1.8
2-propenyl isothiocyanate	1.2
Pyridine	0.8
Tetradecane	7.2
Toluene	2.5
Triacetin	0.4
1,3,5-Trifluorobenzene	2.6
a,a,a-Trifluorotoluene	3.6
1,3,5-trimethyl-Benzene (Mesitylene)	3.6
<i>n</i> -Undecane	5.7
undecan-2-one	3.7
Xylene	3.1

In one embodiment, the encapsulatable material is a pharmaceutically active compound. The pharmaceutically active compound may be in the form of a pro-drug. Pro-drugs may be any covalently bonded carrier that releases a compound in vivo when such pro-drug is administered. Pro-drugs are typically prepared by modifying functional groups in a way such that the modification is cleaved, preferably in vivo, yielding the parent pharmaceutically active compound.

The pharmaceutically active compound may comprise a peptide. The composition may additionally comprise an enzyme inhibitor to mitigate the loss of efficacy of peptide drugs via proteinases. Pharmaceutically active peptide compounds are well publicised as having low absorption properties in the G.I. tract, i.e. low bioavailability.

Illustrative categories and specific examples of pharmaceutically active compounds useful in conjunction with the present invention include: anti-viral agents, analgesics, anesthetics, anti-arthritics, anti-depressants, anti-diabetic agents, anti-inflammatory agents, anti-Parkinsonism drugs, anti-pruritics, cardiovascular drugs, anti-hypertensives, ACE inhibitors, vaccines, hormones, immunosuppressives, muscle relaxants, parasympatholytics, parasympathomimetics, psychostimulants, anti-tuberculosis agents, anti-tussives, histamine H1-receptor antagonists, histamine H2- receptor antagonists, decongestants, alkaloids, mineral supplements, laxatives, vitamins, antacids; ion exchange resins anti-lipidic agents, anti-pyretics, non steroidal anti-inflammatory (NSAI) substances, NSAI oxicam derivatives and appetite suppressants.

Additional useful active medicaments include coronary dilators, cerebral dilators, peripheral vasodilators, anti-infectives, psychotropics, anti-manics, stimulants, gastro-intestinal sedatives and bandages, anti-diarrhoeal and anti-constipation preparations, anti-anginal drugs, vasodilators, anti-hypertensive drugs, vasoconstrictors and migraine treatments, antibiotics, tranquillisers, anti-psychotics, anti-tumour drugs, anti-coagulants, and anti-thrombotic drugs, hypnotics, sedatives, anti-emetics, anti-nauseants, anti-convulsants, neuromuscular drugs, hyper- and hypoglycaemic agents, thyroid and anti-thyroid preparations, diuretics, anti-spasmodics, uterine relaxants, nutritional additives, anti-obesity drugs, anabolic drugs, erythropoietic drugs, anti-asthmatics, anti-histaminic or anti-cholinergic or opiate derivatives, cough suppressants, oral

mucolytics, anti-uricemic drugs and the like. Other examples of actives are well known to a person skilled in the art.

Accordingly, the composition of the present invention may be formulated as a dry or liquid (emulsion or suspension) syrup, a sachet, a chewable, a chewing gum, an orodispersible, a dispersible effervescent, a dispersible tablet, a compressed buccal tablet, a compressed sublingual tablet, a chewable tablet, a melt-in-the-mouth, a lozenge, a paste, a powder, a gel, a tablet, a compressed sweet, a boiled sweet, a cream, a suppository, a snuff, a spray, an aerosol, a pessary, or an ointment.

In another embodiment, the encapsulatable material may be a pesticidal compound. The term "pesticidal compound" as used herein is meant to include any compound capable of adversely affecting normal functioning of an agricultural pest, such as an insect, arachnid, gastropod, mites, ticks, rodents and/or a nematode.

The pesticidal compound may be a peptide or polypeptide. The pesticidal compound may comprise an enzyme. The composition may additionally comprise an enzyme inhibitor to mitigate the loss of efficacy of the enzyme via proteinases.

Illustrative categories and specific examples of pesticidal compounds useful in conjunction with the present invention include: chlorinated hydrocarbons (e.g., DDT), carbamates (e.g., carbaryl), organophosphates (e.g., malathion), pyrethroids (e.g., cypermethrin), insect growth regulators (e.g., diflubenzuron and methoprene), benzoylurea, organochlorides, oximecarbamates, nicotinoids, pyrazole (acaricide), insecticide synergists, e.g. metabolism disrupters and rodenticides (eg. coumarins) . Other examples of actives are well known to a person skilled in the art.

The encapsulatable material may be a biocidally active compound. The term "biocidally active compound" as used herein is meant to include any compound capable of adversely affecting normal functioning of a microbe.

The biocidally active compound may be a fungicide and/or a bactericide, such as, for example antibiotics etc. The biocidally active compound may be selected from phenols and cresols, acids and esters, alkalis, chlorine release agents, iodine compounds, quaternary ammonium compounds, biguanides, diamidines, aldehydes, alcohols, heavy metal derivatives, vapour phase disinfectants, sulphates and nitrites, for example.

The biocidally active compound may comprise one or more essential oils. Essential oils are complex mixtures of odorous, steam volatile or extractable organic compounds, which are synthesised by many types of plant. Essential oils



can be found in various parts of a plant, such as the leaves, stem, flowers, cell organelles, fruit, roots, seeds and bark etc. Generally, the principal constituents are aromatic compounds. Each oil may comprise 100-300 compounds

Essential oils most abundant components include one or more Mono-, di- and sesqui- terpenoids (mevalonic acid derived constituents); phenylpropanoids; alkanes (and alkane derivatives, such as alcohols, aldehydes, and carboxylic acids), alkenes, alkynes and derivatives thereof.

Essential oils are typically mixtures of organic aromatic and other compounds that are extractable from plant material by methods such as steam distillation, cold pressing, CO<sub>2</sub> extraction or extraction with organic solvents or any other means known to the person skilled in the art.

Essential oils for use in the present invention include but are not limited to extracts from Bay (*Pimenta recemosa*); Bergamot (*Citrus bergamia*); Cardamom (*Elettaria cardamom*); Cedarwood (*Cedrus deodara* and *Juniperus virginiana*); Cinnamon leaf (*Cinnamomum zellanicum* Ceylon); Clove or clove bud (*Eugenia caryophyllata* Madagascar extra; *Syzygium aromaticum* L./*Eugenia aromaticum* L.); Cumin seed (*Cuminum cyminum*); Eucalyptus (*Eucalyptus globulus* & *radiata*); Geranium (*Pelargonium graveolens* Madagascar bourbon); Grapefruit (*Citrus paradisi*); Lavender (*Lavendula officinalis* France); Lemongrass (*Cymbopogon citrates*); Manuka (*Leptospermum scoparium*); Marjoram

(*Origanum majorana*); Origanum (*Origanum vulgare*); *Cymbopogon martini*); Palmarosa (*Origanum heracleoticum*); Patchouli (*Pogostemon cablin* E. India dark); Peppermint (*Mentha piperita*); Rosemary (*Rosmarinus officinalis*); Rosewood (*Aniba rosaeodora*); Sage (*Salvia triboia*); Sandalwood (*Aniba rosaeodora*); Savory (*Satureia thymbra*); Tea Tree (*Melaleuca alternifolia*)/*Leptospermum petersonii*); Thyme (*Thymus capitatus*). Other essential oils useful in the present invention include Sandal oil, KapurTulsi oil, and Ropan oil.

Preferably, compositions according to the present invention comprise one or more essential oils from the group comprising Manuka, Geranium, Lavender, Lemongrass, Tea tree and Rosewood oil. More preferably, the compositions of the present invention comprise two or more essential oils selected from the group comprising Manuka, Geranium, Lavender, Lemongrass, Tea tree and rosewood. More preferably still, the composition of the present invention comprises one or more of the following combinations of essential oils; Rosewood +Manuka, Rosewood + Lemongrass, Rosewood + Geranium, Rosewood + Lavender, Rosewood + Tea tree, Manuka + Lemongrass, Manuka + Geranium, Manuka + Lavender, Manuka + Tea tree, Lemongrass + Tea tree, Lemongrass + Lavender, Lemongrass + Geranium, Geranium + Lavender, Geranium + Tea tree and Lavender and Tea tree.

Other common chemical constituents of essential oils are citral (geranial and neral isomers), limonene, linalyl acetate and estragole (methyl chavicol), mono-, sesqui- and di-terpenoids (mevalonic acid-derived constituents); phenylpropanoids (cinnamic acid-derived compounds) and alkane derivatives (alkanes, alkenes, alkynes, alkanols, alkanals, alkanoic acids: mostly acetogenins).

It is understood that the term "essential oil" as used herein includes the naturally occurring extractable plant oils, mixtures thereof, or one or more of the components found in extractable plant oils, whether naturally or artificially synthesized. The term also includes derivatives and analogues of the components found in extractable plant oils.

The composition preferably contains a biocidally active compound in an amount effective to inhibit the growth of a pathogen on a surface to which the composition is applied. The active ingredient is preferably present in the composition in an amount such that when the composition is applied to a surface, the active ingredient is preferably present in an amount of from about 5 to about 30  $\mu\text{g}/\text{cm}^2$  on or over said surface.

The biocidally active compound may comprise an essential oil and/or any one or more of econazole, triclosan, rifampicin and mupirocin.

In one composition, the fungicide is econazole.

In another embodiment, the biocidally active compound may be triclosan (obtainable from Cambiochem California, USA of EMD Biosciences Inc., an affiliate of Merck, Germany

The encapsulatable material preferably has a positive partition coefficient ( $\text{LogP}_{o/w}$ ) greater than 0.1, more preferably in the range 0.1-10, even more preferably, 0.5 - 10, even more preferably still 0.5-7.0, most preferably 2.0-7.0.

The encapsulatable material may have a pH in the range pH1.0 –12.0, preferably pH4-9.

Preferably the encapsulatable material is not acidic or basic in nature but if it is acid it should have a pKa between 2.0-7.0, most preferably between 4.0-7.0. If basic it should have a pKa between 7.0-12, most preferably between 7.0-10.0.

Preferably the encapsulatable material is a liquid at s.t.p (20 °C, 1 atm.) or dissolved in an organic solvent. Preferably the biocidally active compound is soluble in the carrier at a level above 10g/l, preferably above 100 g/l, most preferably above 500 g/l.

Preferably, the encapsulatable material is present in an amount from 1-50 g/100g of product.

The composition may be formulated as solutions, emulsions, suspensions, powders, foams, pastes, granules, aerosols, active-compound-impregnated natural and synthetic materials, very fine encapsulations in polymeric substances and in coating compositions for seeds, furthermore in formulations with smokes, such as fumigating cartridges, fumigating cans, fumigating coils and the like, and also ULV cold mist and warm mist formulations.

These formulations are prepared in a known manner, for example by mixing the composition, with extenders, that is, liquid solvents, pressurized liquefied gases and/or solid carriers, optionally with the use of surface-active agents, that is, emulsifying agents and/or dispersing agents, and/or foam-forming agents. In the case of the use of water as an extender, organic solvents can, for example, also be used as auxiliary solvents. As liquid solvents, there are suitable in the main: aromatics, such as xylene, toluene or alkylnaphthalenes, chlorinated aromatics or chlorinated aliphatic hydrocarbons, such as chlorobenzenes, chloroethylenes or methylene chloride, aliphatic hydrocarbons, such as cyclohexane or paraffins, for example mineral oil fractions, alcohols, such as butanol or glycol as well as their ethers and esters, ketones, such as acetone, methyl ethyl ketone, methyl isobutyl ketone or cyclohexanone, strongly polar solvents, such as dimethylformamide and dimethyl sulphoxide, as well as water; by liquefied

gaseous extenders or carriers are meant liquids which are gaseous at ambient temperature and under atmospheric pressure, for example aerosol propellant, such as halogenated hydrocarbons as well as butane, propane, nitrogen and carbon dioxide; as solid carriers there are suitable: for example ground natural minerals, such as kaolins, clays, talc, chalk, quartz, attapulgite, montmorillonite or diatomaceous earth, and ground synthetic minerals, such as finely divided silica, alumina and silicates; as solid carriers for granules there are suitable: for example crushed and fractionated natural rocks such as calcite, marble, pumice, sepiolite and dolomite, as well as synthetic granules of inorganic and organic meals, and granules of organic material such as sawdust, coconut shells, maize cobs and tobacco stalks; as emulsifying and/or foam-forming agents there are suitable: for example nonionic and anionic emulsifiers, such as polyoxyethylene fatty acid esters, polyoxyethylene fatty alcohol ethers, for example alkylaryl polyglycol ethers, alkylsulphonates, alkyl sulphates, arylsulphonates as well as protein hydrolysates as dispersing agents there are suitable: for example lignin-sulphite waste liquors and methylcellulose. Additionally, other adjuvants including natural compounds such as essential oils, their derivatives and synthetic analogues.

Adhesives such as carboxy-methylcellulose and natural and synthetic polymers in the form of powders, granules or latices, such as gum arabic, polyvinyl alcohol and polyvinyl acetate, as well as natural phospholipids, such as cephalins and

lecithins, and synthetic phospholipids, can be used in the formulations. Further additives may be mineral and vegetable oils.

It is possible to use colourants such as inorganic pigments, for example iron oxide, titanium oxide and Prussian Blue, and organic dyestuffs, such as alizarin dyestuffs, azo dyestuffs and metal phthalocyanine dyestuffs, and trace nutrients such as salts of iron, manganese, boron, copper, cobalt, molybdenum and zinc.

Garlic oil (ex. Firmenich) was encapsulated in washed ethanol yeast from Biofuel (*Saccharomyces cerevisiae*). Garlic oil was encapsulated to 33% w/w using a ratio of 1 part flavour to 2 parts yeast (in a yeast slurry/solution of 30% D/S); the encapsulation was performed under constant agitation of at least 500rpm. Preferably the agitation for encapsulation is completed using a high shear mixer, more preferable a propeller or impellor more preferably still a flat blade stirrer. Preferably the encapsulation is completed at 50-60°C, more preferably at 30-40°C or more preferably still at 40-50°C.

The encapsulation is completed for at least 10 minutes and more preferably 1-24 hours and more preferably still 4-5 hours

In accordance with a further aspect of the present invention, there is provided a method of producing a microbially encapsulated exogenous material, wherein the microcapsule comprises an autolysed microbial cell and the method comprises

the steps of contacting the autolysed microbial cell with the encapsulatable material such that the encapsulatable material is substantially encapsulated within the autolysed microbial cell.

In accordance with a further aspect of the present invention, there is provided a method of encapsulation comprising the steps of:

- a) admixing a microbial cell with an exogenous compound such that at least some of the exogenous compound is encapsulated; and
- b) admixing an autolysed microbial cell with the admixture to encapsulate any non-encapsulated exogenous material.

Thus the microbial cell wall can be used for direct encapsulation of lipophilic active ingredients or used to encapsulate the active ingredient left outside yeast cells after the methods described in the prior art have been completed (so-called 'mop up' agent), thereby improving the efficiency of uptake of the active ingredient used. Yeast cell walls will also take up water soluble and water insoluble materials in the same ratios at which they are exposed.

In accordance with a further aspect of the present invention, there is provided a composition comprising a microcapsule and an encapsulatable material, wherein the microcapsule comprises a microbial cell having a cell wall and at least 30 % carbohydrate by dry weight, and wherein the encapsulatable material is substantially encapsulated within the cell wall.



The microcapsules of the present invention may comprise any one or more selected from processed yeast cells, further processed yeast cells, yeast cell walls and yeast ghost cells.

Specific embodiments of the present invention will now be described, by way of example only, with reference to the following examples and figures, in which:

Fig.1 illustrates the biochemical differences between yeast cells, processed yeast cells, further processed yeast cells, yeast cell walls and ghost cells.

Fig.2 illustrates the production and process differences between yeast cells, processed yeast cells, further processed yeast cell walls and ghost cells.

Fig.3 illustrates the formulation and encapsulation differences between yeast cells, processed yeast cells, further processed yeast cell wall and ghost cells.

Fig. 4 is a graph showing the percentage encapsulation of carbinol against time at 20 °C and 60 °C for the release of carbinol in water from baker's yeast and a composition according to the present invention added at 4 hours;

Fig. 5 is a graph showing the percentage encapsulation of carbinol against time at 20 °C and 60 °C for the release of carbinol in water from a composition according to the present invention;

Fig. 6 is a bar graph illustrating the results of encapsulation concentration and encapsulation efficiency for processed yeast cells, further processed yeast cells, extremely processed yeast cells and Baker's yeast and a biofuel yeast;

Fig. 7 is a graph illustrating the release of a fragrance from processed yeast cells and further processed yeast cell walls at varying temperatures;

Figs. 8a – f are electronmicrograph images of processed yeast cells yeast when viewed using scanning electron microscope (SEM)

Figs. 9 a - d are electronmicrograph images of further processed yeast when viewed using scanning electron microscope (SEM)

Figs. 10 a – d are electronmicrograph images of extremely processed yeast when viewed using scanning electron microscope (SEM)

Figs. 11 a – d are electronmicrograph images of biofuel yeast and baker's yeast when viewed using scanning electron microscope (SEM)

Fig. 12 is a bar graph illustrating the encapsulation efficiency of a method according to the present invention for improving the efficiency of encapsulation.

Figs. 1, 2 and 3 illustrate whole yeast cells, washed Biofuel yeast, processed yeast cells, further processed yeast cells and extremely processed yeast cells. Whole, intact live yeast cells primarily in this document live bakers yeast has an approximate biochemical "make up" of 50% protein, 35% carbohydrate, 8% lipid, 4% ash and 4% moisture. Washed Biofuel yeast is a dead relatively intact yeast with a biochemical "make up" of 45% protein, 30% carbohydrate, 10% lipid, 8% ash and 5% moisture. Processed yeast cells are dead yeast cells that have had a proportion of the internal cell contents removed via gentle digestion. The biochemical "make up" of these processed yeast cells are 30% protein, 51% carbohydrate, 11% lipid, 3% ash and 5% moisture. Further processed yeast also

called "ghost cells" in this document yeast cells that have been further processed from the processed yeast cells. The biochemical "make up" of these further processed yeast cells are 8% protein, 78% carbohydrate, less than 1% lipid, 10% ash and 5% moisture. Extremely processed yeast cells also called yeast cell walls in this document are yeast cells that have been processed further than the ghost cells. The biochemical "make up" of these extremely processed yeast cells are 6.5% protein, 85% carbohydrate, less than 1% lipid, 9% ash and 1% moisture. The following table provides a comparison of the biochemical make up by dry weight of each yeast cell type

Yeast	Protein	Carbohydrate	Lipid	Ash	Moisture
Whole yeast	50%	35%	8%	4%	4%
Biofuel yeast	45%	30%	10%	8%	5%
Processed yeast cells	30%	51%	11%	3%	5%
Further processed yeast cells	8%	78%	<1%	10%	5%
Extremely processed yeast cells	6.5%	85%	<1%	9%	1%

**Example 1: Use of processed yeast cells as a "mop up agent" and as an encapsulation medium in its own right**

**Method**

Carbinol was encapsulated into bakers (*S.cerevesiae*) yeast with yeast cell walls added after the 4 hours encapsulation period. This sample was then spray dried

and the efficiency of flavour usage calculated before the sample was tested for release in water at 20°C and 60°C.

Processed yeast cells were used to encapsulate carbinol, the sample was spray dried and the efficiency of flavour usage calculated before the sample was tested for release in water at 20°C and 60°C for 4 hours.

Simple example of Micap using processed yeast cells to mop up excess flavour when a simple baking yeast has been used first .

Processed yeast cells were then used in their own right to encapsulate carbinol (carbinol is 1-octen-3-ol, mushroom flavour (available from Firmenich SA, Rue de le Bergere 7, CH-1217, Meyrin 2, Geneva, Switzerland)

#### **Yeast cell walls used to take up excess flavour**

Encapsulation of carbinol in bakers yeast with yeast cell walls added after the encapsulation process to take up any excess flavour was completed following the method below.

The pressed yeast cake (200g) was added to distilled water (50g). Carbinol (29.86g) was added to the yeast slurry and the flavour encapsulated for 4 hours at 40°C. After 4 hours yeast cell walls were added (35g) in a suspension of

distilled water (70g). The sample was mixed for 20 minutes more preferably 40 minutes and more preferably still 60 minutes, but can be mixed for 20 minutes to 24 hours until homogenous and then spray dried

### **Encapsulation in yeast cell walls**

The encapsulation of carbinol was performed using processed yeast cells following the method below.

Processed yeast cells (60g) were added to distilled water (240g). Carbinol (24.99g) was added to the yeast cell wall slurry and the flavour encapsulated for 4 hours at 40°C. This sample was then spray dried without any separation or washing step.

Both samples were dried using a Buchi lab spray drier at an inlet temperature of 200°C and an outlet temperature of 100°C.

### **Release of encapsulated carbinol**

Spray dried products 1- and 2 (10g) were suspended in distilled water (80g) and agitated at 100 rpm for 4 hours. Samples were tested for release at 20°C and 60°C. Samples were analyzed after 4 hours for total remaining flavour, i.e.

samples removed, centrifuged, washed (to remove any surface material) and the flavour extracted and analyzed via GC (using current extraction method).

## Results

Fig. 4 shows the release of carbinol from bakers yeast that had yeast cell walls added after the 4 hour encapsulation period. As you can see the encapsulation concentration initially was 26.3% w/w. At 20°C the encapsulated concentration has not decreased (the figure shows an increase however we can put that down to analytical and experimental error). At 60°C there is a small release of carbinol from 26.3% w/w to 18.2% w/w a release of 8.1% w/w.

The release of carbinol in this sample could be attributed to the product encapsulated in the yeast cell walls.

As Fig. 5 shows there is no release of carbinol from the processed yeast cells in water at 20°C. However at 60°C there is a loss of approximately 10% w/w of carbinol flavour from the process yeast cells. The processed yeast cells have encapsulated carbinol to 25% w/w and show no release at 20°C in water which make them a valuable addition to any encapsulation portfolio.

The processed yeast cells potentially has an intact cell membrane which is slightly damaged at elevated temperatures; once the membrane is hydrated this damage to the membrane allows some release of the flavour.

**Encapsulation in Biofuel yeast**

Carbinol (g)	Product (g)	Encapsulation % w/w	Carbinol (g)	Flavour efficiency %
100	132	26	32	32

**Process efficiency using processed yeast cells as an independent encapsulation media and as a "mop up"**

Yeast + yeast cell wall addition				
Carbinol (g)	Product (g)	Encapsulation % w/w	Carbinol (g)	Flavour efficiency
29.86g	66.7g	26.25% w/w	24g	80%
Yeast cell walls				
Carbinol (g)	Product (g)	Encapsulation % w/w	Carbinol (g)	Flavour efficiency
24.99g	64.98g	25.15%	16.34g	64.40%

The above tables shows the flavour efficiency of using processed yeast cells to reclaim excess flavour and the efficiency of using processed yeast cells in the encapsulation of carbinol. Processed yeast cells can be twice as efficient as yeast.

As you can see in table 1 the flavour efficiency of using yeast cell walls after the encapsulation has finished was 80% (for the encapsulation of carbinol). If we consider report 46 (appendix) we have demonstrated that the process of the present invention in bakers yeast cake is approximately 62% efficient in relation

to the carbinol flavour use. The addition of Maltodextrin (DE20 preferably DE10, corn starch or preferably potato starch) after the encapsulation is complete allows 90% of the carbinol to be utilized (see report Micap 46 in appendix 1??). Using yeast cell walls (processed yeast cells) instead of maltodextrin has allowed us to utilize 80% of the flavour and gives the possibility of a better performing product in water.

Processed yeast cells (damaged yeast cells, ghost cells, cell walls) can be as efficient as whole (?) yeast cells for this flavour but appear to lose more flavour in water at 60°C than intact whole yeast.

## Conclusions

The present invention provides an alternative to maltodextrin in mopping up excess flavour from known processes such as that described in EP0242135. The compositions of the present invention also provide improved flavour efficiencies (80%) and improved retention in water.

Using compositions of the present invention to be more efficient with flavours instead of maltodextrin is a means of increasing product performance while being efficient. Using the present compositions instead of whole yeast cells is furthermore advantageous in applications where "nose" is required such as in perfume and fragrance applications.



Using the methods of the present invention in conjunction with yeast increases the encapsulation efficiency of an encapsulatable material and provides a much more commercially viable process.

**Example 2: Encapsulated fragrance in 3 different further processed yeast cells (processed yeast cells, ghost cells, cell walls) and release in water at a range of temperatures**

**Method**

The 3 processed yeast cells of the present invention were used to encapsulate a general fragrance, the recipe used to encapsulate in processed yeast cells was 1 part fragrance to 2 parts yeast cell walls to 8 parts water e.g. 50g fragrance, 100g of yeast cell walls and 400g of distilled water. More water was added to the recipe used for the further processed yeast cells, 1:2:10, and for the extremely processed yeast cells a recipe of 50g of flavour and 100g of extremely processed yeast cell walls and 1000g of distilled water was used. This extra water was added to make the encapsulation mixture homogenous in order for encapsulation to occur.

The encapsulations were completed at 40°C for 4 hours and then the samples were spray dried at 210°C inlet 90°C outlet using a Niro mobile minor.

**Results of encapsulation concentration and encapsulation efficiency**

The three different yeast cell walls, processed, further processed and extremely processed yeast cells are used here to encapsulate a fragrance (in their own right) with no extra yeast added. As you can see the processed yeast cells is much more efficient in active encapsulation than a standard bakers yeast.

Start			Finish	
Fragrance (g)	Yeast (g)	Water (g)	Fragrance (g)	Fragrance Efficiency (%)
50	100	250	20	40

**Example 3: Encapsulation of several savory flavours using HCT yeast cell walls as a “mop up agent”**

Processed yeast cells were used to mop up any excess flavour after the encapsulation of a range of savory flavours in washed Biofuel yeast. This addition of yeast cell walls (processed, further or extremely processed yeast cells) is termed as a 40% addition or a 20% addition because we add 100g of yeast, 100g of yeast cell walls and 50g of flavour or 100g of yeast, 50g of flavour and 50g of yeast cell walls. Therefore the spray dried sample of the 40% addition is 40% yeast, 40% yeast cell walls and 20% flavour and for the 20% yeast cell wall addition its 60% yeast 20% flavour and 20% yeast cell walls.

The encapsulation of encapsulatable material, such as an active, is completed using a recipe of 1 part active to 2 parts yeast to 4 parts water, yeast concentration in the dispersion from 10% - 45%. Microbes such as bacteria, yeast or fungi (washed + Pre processed yeast and/or bacteria) are mixed with water until a homogenous dispersion is formed. The actives are then added and the encapsulation mixture stirred at 40°C of 10-70°C for 10 minutes – 24 hours for 4 hours before addition of “mop up material”

The addition of materials to the “mop up” Yeast cell walls such as yeast cell walls (processed, further or extremely processed yeast cells), de-odorized yeast (extremely processed bleached yeast), yeast cell fragments (ex. Bio-Springer, Lesaffre), whole yeast (active and inactive dried bakers yeast, active and inactive yeast cake, active and inactive yeast cream), any autolysed yeast (e.g. autolysed yeast ex.Quest, ex.Chemoforma / Probio), bacteria both active and inactive (preferably in a dried form). Maltodextrin (potato or corn, starch (capsul), gum (Arabic) etc, solutions of these materials can be made from 1-45% solids and added to the “initial encapsulation”.

Samples are mixed in for a period of 10 minutes – 24 hours preferably 1 hour at 40°C (temperature range of 10-70°C) using (high shear mixer, propeller mixer, preferably flat blade stirrer) and the samples spray dried (preferably) at 210°C inlet 90° outlet (range of 165°C inlet – 120°C outlet) can be dried via fluidized bed drier, roller drier, freeze drier, (spray drying via preferably rotary atomosier, can

be high pressure nozzle, 2 fluid nozzle), can be dried on a box drier and other forms of drying where the material is dehydrated to form a powder. Particle sizes of the material can vary from 10um to 1000um (if agglomerated) standard is 30-50um on lab based spray driers, 100um in industrial spray driers, 300-400um if agglomerated on a fluidized bed drier.

**Example 5: Further processed yeast cells 40% addition to Biofuel yeast during the encapsulation of crispy bacon flavour**

The encapsulation of crispy bacon (ex.Firmenich) was completed using a recipe of 1 part flavour to 2 parts yeast to 4 parts water. Washed ethanol yeast (ex.Biofuel 100g) was mixed with 220g of water until a homogenous dispersion was formed. Crispy bacon flavour (50g) was then added and the encapsulation mixture was then stirred at 40°C for 4 hours before the addition of further processed yeast cells.

Processed yeast cells (100g) were mixed until homogenous with 600g of distilled water and then this yeast cell wall solution was added to the yeast encapsulation mixture. The sample was then mixed for a further 60 minutes at 40°C and then spray dried at 210°C inlet 90°C outlet using a Niro mobile minor.

Of the 250g of material for spray drying 216.9g of product was dried resulting in a total process efficiency of 87%. The encapsulation concentration was 14.1%

resulting in 31g of flavour being utilised a flavour efficiency for this process of 62%.

**Example 6: Processed yeast cells 20% addition to Biofuel yeast during the encapsulation of crispy bacon**

The encapsulation of crispy bacon (ex.Firmenich) was completed using a recipe of 1 part flavour to 2 parts yeast to 4 parts water. Washed ethanol yeast (biofuel yeast) (100g) was mixed with 220g of water until a homogenous dispersion was formed. Crispy bacon flavour (50g) was then added and the encapsulation mixture was then stirred at 40°C for 4 hours before the addition of processed yeast cells.

Processed yeast cells (50g) were mixed until homogenous with 300g of distilled water and then this processed yeast cell solution was added to the yeast encapsulation mixture. The sample was then mixed for a further 60 minutes at 40°C and then spray dried at 210°C inlet 90°C outlet using a Niro mobile minor.

Of the 200g of material for spray drying 189.7g of product was dried resulting in a total process efficiency of 94.85%. The encapsulation concentration was 17.2% resulting in 32.3g of flavour being utilized a flavour efficiency for this process of 65%.

**Example 7: Processed yeast cells 40% addition to Biofuel yeast during the encapsulation of epices tandori**

The encapsulation of epices tandori (ex.Firmenich) was completed using a recipe of 1 part flavour to 2 parts yeast to 4 parts water. Washed ethanol yeast (ex.Biofuel 100g) was mixed with 220g of water until a homogenous dispersion was formed. Epices Tandori flavour (50g) was then added and the encapsulation mixture was then stirred at 40°C for 4 hours before the addition of processed yeast cells.

Processed yeast cells (100g) were mixed until homogenous with 600g of distilled water and then this processed yeast cell (yeast cell wall) solution was added to the yeast encapsulation mixture. The sample was then mixed for a further 60 minutes at 40°C and then spray dried at 210°C inlet 90°C outlet.

Of the 250g of material for spray drying 208.65g of product was dried resulting in a total process efficiency of 83.46%. The encapsulation concentration was 18% resulting in 37.6g of flavour being utilised a flavour efficiency for this process of 75.1%.

**Example 8: Processed yeast cells 20% addition to Biofuel yeast during the encapsulation of epices tandori**

The encapsulation of epices tandori (ex.Firmenich). was completed using a recipe of 1 part flavour to 2 parts yeast to 4 parts water. Washed ethanol yeast (100g) was mixed with 220g of water until a homogenous dispersion was formed. Epices tandori flavour (50g) was then added and the encapsulation mixture was then stirred at 40°C for 4 hours before the addition of processed yeast cells.

Processed yeast cells (50g) were mixed until homogenous with 300g of distilled water and then this processed yeast cell solution was added to the yeast encapsulation mixture. The sample was then mixed for a further 60 minutes at 40°C and then spray dried at 210°C inlet 90°C outlet.

Of the 200g of material for spray drying 173.9g of product was dried resulting in a total process efficiency of 86.95%. The encapsulation concentration was 17.4% resulting in 30.2g of flavour being utilized a flavour efficiency for this process of 60.5%.

**Example 9: Processed yeast cells 40% addition to Biofuel yeast during the encapsulation of BBQ flavour**

The encapsulation of BBQ flavour (ex.Firmenich) was completed using a recipe of 1 part flavour to 2 parts yeast to 4 parts water. Washed ethanol yeast (100g) was mixed with 220g of water until a homogenous dispersion was formed. BBQ

flavour (50g) was then added and the encapsulation mixture was then stirred at 40°C for 4 hours before the addition of processed yeast cells.

Processed yeast cells (100g) were mixed until homogenous with 600g of distilled water and then this processed yeast cell (cell wall) solution was added to the yeast encapsulation mixture. The sample was then mixed for a further 60 minutes at 40°C and then spray dried at 210°C inlet 90°C outlet.

Of the 250g of material for spray drying 203.25g of product was dried resulting in a total process efficiency of 81.30%. The encapsulation concentration was 19.1% resulting in 38.8g of flavour being utilised a flavour efficiency for this process of 77.64%.

**Example 10: Processed yeast cells 20% addition to Biofuel yeast during the encapsulation of BBQ flavour**

The encapsulation of BBQ (ex.Firmenich) was completed using a recipe of 1 part flavour to 2 parts yeast to 4 parts water. Washed ethanol yeast (100g) was mixed with 220g of water until a homogenous dispersion was formed. BBQ flavour (50g) was then added and the encapsulation mixture was then stirred at 40°C for 4 hours before the addition of processed yeast cells.



Processed yeast cells (50g) were mixed until homogenous with 300g of distilled water and then this processed yeast cell (cell wall) solution was added to the yeast encapsulation mixture. The sample was then mixed for a further 60 minutes at 40°C and then spray dried at 210°C inlet 90°C outlet.

Of the 200g of material for spray drying 179.88g of product was dried resulting in a total process efficiency of 89.84%. The encapsulation concentration was 19% resulting in 34.3g of flavour being utilized a flavour efficiency for this process of 70%.

**Example 11: Processed yeast cells 40% addition to Biofuel yeast during the encapsulation of roast garlic**

The encapsulation of roast garlic (ex.Firmenich) was completed using a recipe of 1 part flavour to 2 parts yeast to 4 parts water. Washed ethanol yeast (100g) was mixed with 220g of water until a homogenous dispersion was formed. Roast garlic flavour (50g) was then added and the encapsulation mixture was then stirred at 40°C for 4 hours before the addition of processed yeast cells.

Processed yeast cells (100g) were mixed until homogenous with 600g of distilled water and then this processed yeast cell (cell wall) solution was added to the yeast encapsulation mixture. The sample was then mixed for a further 60 minutes at 40°C and then spray dried at 210°C inlet 90°C outlet.

Of the 250g of material for spray drying 248.25g of product was dried resulting in a total process efficiency of 99.3%. The encapsulation concentration was 20% resulting in 49g of flavour being utilised a flavour efficiency for this process of 99%.

**Example 12: Processed yeast cells 20% addition to Biofuel yeast during the encapsulation of roast garlic**

The encapsulation of roast garlic (ex.Firmenich) was completed using a recipe of 1 part flavour to 2 parts yeast to 4 parts water. Washed ethanol yeast (100g) was mixed with 220g of water until a homogenous dispersion was formed. Roast garlic flavour (50g) was then added and the encapsulation mixture was then stirred at 40°C for 4 hours before the addition of processed yeast cells.

Processed yeast cells (50g) were mixed until homogenous with 300g of distilled water and then this processed yeast cell (yeast cell wall) solution was added to the yeast encapsulation mixture. The sample was then mixed for a further 60 minutes at 40°C and then spray dried at 210°C inlet 90°C outlet.

Of the 200g of material for spray drying 192g of product was dried resulting in a total process efficiency of 96%. The encapsulation concentration was 20%.

resulting in 38.4g of flavour being utilized a flavour efficiency for this process of 76.8%.

**Example 13: Processed yeast cells 40% addition to Biofuel yeast during the encapsulation of Nonanol a single component flavour**

The encapsulation of nonanol was completed using a recipe of 1 part flavour to 2 parts yeast to 4 parts water. Washed ethanol yeast (100g) was mixed with 220g of water until a homogenous dispersion was formed. Nonanol (50g) was then added and the encapsulation mixture was then stirred at 40°C for 4 hours before the addition of processed yeast cells.

Processed yeast cells (100g) were mixed until homogenous with 600g of distilled water and then this processed yeast cells (cell wall) solution was added to the yeast encapsulation mixture. The sample was then mixed for a further 60 minutes at 40°C and then spray dried at 210°C inlet 90°C outlet.

Of the 250g of material for spray drying 231g of product was dried resulting in a total process efficiency of 92.4%. The encapsulation concentration was 18% resulting in 41.58g of flavour being utilised a flavour efficiency for this process of 83.16%.

**Example 14: Processed yeast cells 40% addition to Biofuel yeast during the encapsulation of ibuprofen using benzyl alcohol as a carrier**

The encapsulation of ibuprofen was completed using a recipe of 1 part ibuprofen and benzyl alcohol (40% ibuprofen and 60% benzyl alcohol) to 2 parts yeast to 4 parts water. Washed ethanol yeast (100g) was mixed with 220g of water until a homogenous dispersion was formed. Ibuprofen and benzyl alcohol (50g) was then added and the encapsulation mixture was then stirred at 40°C for 4 hours before the addition of processed yeast cells.

Processed yeast cells (100g) were mixed until homogenous with 600g of distilled water and then this processed yeast cell (cell wall) solution was added to the yeast encapsulation mixture. The sample was then mixed for a further 60 minutes at 40°C and then spray dried at 210°C inlet 90°C outlet.

Of the 250g of material for spray drying 231g of product was dried resulting in a total process efficiency of 92.4%. The encapsulation concentration was 18% resulting in 41.58g of the ibuprofen and benzyl alcohol being utilised an "active" efficiency for this process of 83.16%.

**Example 15: Processed yeast cells 40% addition to the encapsulation of oleic acid using medical yeast *Saccharomyces boulardii***

The encapsulation of oleic acid was completed using a recipe of 1 part oleic acid and to 2 parts yeast to 4 parts water. Medical yeast (ex. DHW 100g) was mixed with 220g of water until a homogenous dispersion was formed. Oleic acid (50g) was then added and the encapsulation mixture was then stirred at 40°C for 4 hours before the addition of processed yeast cells.

Processed yeast cells (100g) were mixed until homogenous with 600g of distilled water and then this processed yeast cell (cell wall) solution was added to the yeast encapsulation mixture. The sample was then mixed for a further 60 minutes at 40°C and then spray dried at 210°C inlet 90°C outlet.

Of the 250g of material for spray drying 189g of product was dried resulting in a total process efficiency of 75.6%. The encapsulation concentration was 14.6% resulting in 27.6g of the oleic acid being utilised an "active" efficiency for this process of 55.2%.

**Example 16: Further processed yeast cells, ghost 40% addition to the encapsulation of Garlic oil using washed ethanol yeast**

The encapsulation of garlic oil was completed using a recipe of 1 part garlic oil and to 2 parts yeast to 4 parts water. Pre-washed ethanol yeast (100g) was mixed with 220g of water until a homogenous dispersion was formed. Garlic oil

(50g) was then added and the encapsulation mixture was then stirred at 40°C for 4 hours before the addition of further processed yeast cells.

Further processed yeast cells (100g) were mixed until homogenous with 700g of distilled water and then this further processed yeast cell (cell wall) solution was added to the yeast encapsulation mixture. The sample was then mixed for a further 60 minutes at 40°C and then spray dried at 210°C inlet 90°C outlet.

Of the 250g of material for spray drying 210g of product was dried resulting in a total process efficiency of 84%. The encapsulation concentration was 21% resulting in 44.1g of the garlic oil being utilised an "active" efficiency for this process of 88.2%.

**Example 17: Extremely processed 40% addition to the encapsulation of Eucalyptus oil using washed bakers yeast**

The encapsulation of eucalyptus oil was completed using a recipe of 1 part eucalyptus oil and to 2 parts yeast to 4 parts water. Pre-washed bakers yeast (ex.Lesaffre 100g) was mixed with 220g of water until a homogenous dispersion was formed. Eucalyptus oil (50g) was then added and the encapsulation mixture was then stirred at 40°C for 4 hours before the addition of extremely processed yeast cells.

Extremely processed yeast cells (100g) were mixed until homogenous with 700g of distilled water and then this extremely processed yeast cell (cell wall) solution was added to the yeast encapsulation mixture. The sample was then mixed for a further 60 minutes at 40°C and then spray dried at 210°C inlet 90°C outlet.

Of the 250g of material for spray drying 210g of product was dried resulting in a total process efficiency of 84%. The encapsulation concentration was 21% resulting in 44.1g of the garlic oil being utilised an "active" efficiency for this process of 88.2%.

**Example 18: Encapsulation of oleic acid using medical yeast (ex.DHW)**

***Saccharomyces boulardii***

The encapsulation of oleic acid was completed using a recipe of 1 part oleic acid and to 2 parts yeast to 4 parts water. Medical yeast (ex.DHW 100g) was mixed with 220g of water until a homogenous dispersion was formed. Oleic acid (50g) was then added and the encapsulation mixture was then stirred at 40°C for 4 hours before being separated via centrifugation and then spray dried.

Of the 150g of material for spray drying 78g of product was dried resulting in a total process efficiency of 52%. The encapsulation concentration was 6.01% resulting in 4.8g of the oleic acid being utilised an "active" efficiency for this process of 9.5%.

**Example 19: Encapsulation of oleic acid using processed yeast cells**

The encapsulation of oleic acid was completed using a recipe of 1 part oleic acid (50g) and 2 parts processed yeast cells (100g) and 600g of distilled water (processed yeast cells are highly hydroscopic and need more water in order to get them to disperse and mix, if the concentration is too high encapsulation will not occur as the active will not reach the surface and will not be sufficiently enough dispersed to encapsulate). Processed yeast cells (100g) are dispersed in water (600g) for 10 minutes – 1 hour (preferably 30 minutes) at 40°C (preferably) (10-70°C would be the range) at 500rpm (range of speeds and mixers) until homogenous. Oleic acid (50g) was then added and sample continuously mixed for 4 hours at 40°C (range of time 10 minutes – 24 hours and temperatures from 10-70°C) before being spray dried (no centrifugation).

Of the 150g of material for spray drying 129g of product was dried resulting in a total process efficiency of 86%. The encapsulation concentration was 11.8% resulting in 15.2g of the oleic acid being utilised an “active” efficiency for this process of 30.4%.

**Example 20: Encapsulation of oleic acid using further processed yeast cells, ghost cells**



The encapsulation of oleic acid was completed using a recipe of 1 part oleic acid (50g) and 2 parts further processed yeast cells (100g) and 650g of distilled water (further processed yeast cells are highly hydroscopic and need more water in order to get them to disperse and mix, if the concentration is too high encapsulation will not occur as the active will not reach the surface and will not be sufficiently enough dispersed to encapsulate). Further processed yeast cells (HGT yeast cell walls) (100g) are dispersed in water (650g) for 10 minutes – 1 hour (preferably 30 minutes) at 40°C (preferably) (10-70°C would be the range) at 500rpm (range of speeds and mixers) until homogenous. Oleic acid (50g) was then added and sample continuously mixed for 4 hours at 40°C (range of time 10 minutes – 24 hours and temperatures from 10-70°C) before being spray dried (no centrifugation).

Of the 150g of material for spray drying 132g of product was dried resulting in a total process efficiency of 88%. The encapsulation concentration was 15.3% resulting in 20.2g of the oleic acid being utilised an "active" efficiency for this process of 40.4%.

**Example 21: Encapsulation of oleic acid using extremely processed yeast cells, cell walls**

The encapsulation of oleic acid was completed using a recipe of 1 part oleic acid (50g) and 2 parts extremely processed yeast cells (100g) and 1000g of distilled

water (extremely processed yeast cells are highly hydroscopic and need more water in order to get them to disperse and mix, if the concentration is too high encapsulation will not occur as the active will not reach the surface and will not be sufficiently enough dispersed to encapsulate). Extremely processed yeast cells (YGT Plus yeast cell walls) (100g) are dispersed in water (1000g) for 10 minutes – 1 hour (preferably 30 minutes) at 40°C (preferably) (10-70°C would be the range) at 500rpm (range of speeds and mixers) until homogenous. Oleic acid (50g) was then added and sample continuously mixed for 4 hours at 40°C (range of time 10 minutes – 24 hours and temperatures from 10-70°C) before being spray dried (no centrifugation).

Of the 150g of material for spray drying 129g of product was dried resulting in a total process efficiency of 86%. The encapsulation concentration was 23.8% resulting in 30g of the oleic acid being utilised an “active” efficiency for this process of 60.1%.

**Modification examples for industrial scale process of flavour/active encapsulation and ‘mop-up’.**

The standard method comprises adding the microcapsules, yeast, to water, add the flavour /active and mix. Maltodextrin or yeast cell walls to mop-up any non-encapsulated active/flavour can then be added to the mix. Additional water is subsequently added to the mixture to allow the mixture to be spray dried:

- 500Kg of washed Biofuel to 1200Kg of water mix until homogenous (40C)
- Add 250Kg of flavour mix for 2 hours at 40C
- Add 500Kg of maltodextrin and then 500Kg of water, mix for 30 mins and spray dry (or add 500Kg of yeast cell walls and 2500Kg of water, mix and dry)

Alternatively, the following method can be used:

- 500Kg of washed Biofuel to 1700Kg of water mix until homogenous (40C)
- Add 250Kg of flavour mix for 2 hours at 40C
- Add 500Kg of maltodextrin, mix and then pass through an in-line high shear Silverson and dry

If it were desired to change the maltodextrin for the yeast cell walls then at the beginning you would add 500Kg of yeast to 3700Kg of water and then 500Kg of yeast cell walls, pass through the in-line Silverson and dry

If it were desired to use a yeast cell wall only then the following method can be used:

- 500Kg of yeast cell walls to 2500Kg of water mix until homogenous (40C)
- Add 250Kg of flavour mix for 2 hours at 40C
- Pass through an in-line Silverson and dry

Maltodextrin or yeast cell walls can then be added to mop-up excess active/ flavour, if desired.

**Comparison of yeast vs. yeast cell walls (active efficiency and total efficiency)**

<b>Yeast Type</b>	<b>Oleic acid concentration</b>	<b>Active efficiency</b>	<b>Total efficiency</b>
medical yeast	6.01% w/w	9.5%	55.2%
processed yeast cell wall	11.8% W/W	30.4%	86%
further processed yeast cell wall	15.27% w/w	40.4%	88%
extremely processed yeast cell wall	23.3% w/w	60.1%	86%

The extremely processed yeast cell wall encapsulates more of the oleic acid (than the other yeast cell walls) than the whole medical yeast.

**Investigation of the yeast cell walls using cryo-SEM**

**Sample preparation.**

A small amount of each sample was quickly mixed with an equal volume of de-ionized water to produce a liquescent paste. Approximately 1mm<sup>3</sup> of paste was immediately placed into the top of a high thermal conductivity specimen holder and quickly quench cooled in melting iso-pentane at 113K. Using the same equipment described in previous experiments, the now frozen sample was allowed to reach thermal stability and fractured at 120K and then immediately sputter coated with 8nm platinum at the same temperature. The sample was

moved to the pre-cooled cold stage of the microscope and allowed to reach thermal stability at 120K. Specimens were examined at between x1000 to x50,000 and photographed at 2-5keV and 15-30pA. The images were recorded as video prints and stored on a CD-ROM.

## **Experimental results**

### Sample CW-1. processed yeast cells

Referring to Figs. 9a – f, recognizable yeast cells could be seen in all the images although the cell contents were very distorted with no recognizable sub-structure. All the yeast cells had a jumble of higher emissive material at the centre surrounded by a featureless peripheral layer. There are some outlines of what appear to be the external cell membrane although there were none of the characteristic images normally associated with these structures. Images T-2, T-3 and T-4 show cell walls although their presence was determined more by location rather than any recognizable sub-structure.

### Sample CW-2. further processed yeast cells.

Referring to Fig. 10 a – d, It proved very difficult to find any recognizable structures which could be positively associated with yeast cells. There are structures which could be described as cell like (see A-09 and A-10) but there are no recognizable cell walls and certainly none of the characteristic yeast sub-structure shown in earlier reports. There were several groups of the structure

shown in image T-16. They are the correct size and shape of yeast cells but they lack a cell wall and are devoid of any identifiable structures. A second sample of this specimen was prepared and examined, but the images were identical to the first.

Sample CW-3. extremely processed yeast cells

Referring to Fig. 11 , it was not possible to identify any yeast like material other than very small (40-50nm) dark electron dense particles on the sample surface. In an attempt to obtain further information, the sample was allowed to freeze dry (Images T-17, T-21 and T-22). The resulting structures could be interpreted as highly distorted yeast cells which curiously, formed groups of structures separated one from another by long skeins of unidentifiable material.

**Cryo-Sem investigation of biofuel yeast and active dried bakers yeast**

Fig. 12 a – d shows the similarities in two yeasts which have been produced for ethanol production and bread making (DCL instant dried active bakers yeast). The different starting materials have been given the same in-house pre-treatments consisting of a washing step and spray drying prior to the encapsulation process. Fig 12 shows pre-processed yeast viewed before and after encapsulation. Orange oil encapsulated in biofuel yeast and Tee Tree essential oil encapsulated in DCL bakers yeast.

CLAIMS

1. A composition comprising a microcapsule and an encapsulatable material, wherein the microcapsule comprises an autolysed microbial cell having a cell wall, wherein the encapsulatable material is substantially encapsulated within the cell wall.
2. A composition as claimed in claim 1 wherein the microcapsule is derived from a fungal cell, bacterial cell or algae cell.
3. A composition as claimed in claim 2 wherein the microcapsule is derived from a fungal cell.
4. A composition as claimed in claim 3 wherein the microcapsule is derived from one or more fungi from the group comprising *Mastigomycotina*, *Zygomycotina*, *Ascomycotina*, *Basidiomycotina* and *Deuteromycotina*.
5. A composition as claimed in claim 4 wherein the microcapsule is derived from one or more fungi from *Ascomycotina*.
6. A composition as claimed in claim 5 wherein the microcapsule is derived from yeasts.

7. A composition as claimed in claim 6 wherein the microcapsule is derived from one or more of the group comprising *Candida albicans*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Paracoccidioides brasiliensis*, *Penicillium marneffe* and *Saccharomyces cerevisiae*.
8. A composition as claimed in claim 7 wherein the microcapsule is derived from *Saccharomyces cerevisiae*.
9. A composition as claimed in claim 8 wherein the microcapsule is derived from bakers yeast
10. A composition as claimed in claim 8 or 9 wherein the microcapsule is derived as a byproduct of ethanol biofuel production.
11. A composition as claimed in any one of the previous claims wherein the cell from which the microcapsule is derived is a ghost cell.
12. A composition as claimed in any one of the previous claims wherein the lipid content of the autolysed cell is less than 60% by dry weight of the microcapsule.
13. A composition as claimed in claim 12 wherein the lipid content of the autolysed cell is less than 40% by dry weight of the microcapsule.



14. A composition as claimed in claim 14 wherein the lipid content of the autolysed cell is less than 25% by dry weight of the microcapsule.
15. A composition as claimed in claim 14 wherein the lipid content of the autolysed cell is less than 15% by dry weight of the microcapsule.
16. A composition as claimed in claim 15 wherein the lipid content of the autolysed cell is less than 5% by dry weight of the microcapsule.
17. A composition as claimed in claim 16 wherein the lipid content of the autolysed cell is less than 1% by dry weight of the microcapsule.
18. A composition as claimed in any preceding claim wherein the autolysed cell comprises at least 70% polysaccharides, at least 80% polysaccharides or at least 90% polysaccharides.
19. A composition as claimed in claim 18 wherein the polysaccharides comprise any one or more of glucans, mannans and chitin.
20. A composition as claimed in claim 19 wherein the glucans comprise  $\beta$ -2,6 and/or  $\beta$ -1,3-linked glucans.

21. A composition as claimed in claim 19 or 20 wherein the comprise a -1,6-linked inner core with a -1,2- or a -1,3 side chain.
22. A composition as claimed in any preceding claim wherein the weight percentage of chitin in the autolysed cell comprises at least 1%, is in the range 1-5% or is in the range 2-4%.
23. A composition as claimed in any preceding claim wherein the autolysed cell has a cell wall thickness of at least 70 nm or 100-200 nm thick
24. A compositionas claimed in any one of the preceding claims wherein the protein content of the autolysed cell wall is less than 50%, less than 45 less than 40%, less than 30%, less than 20% or less than 10% by dry weight of the microcapsule.
25. A composition as claimed in any one of the previous claims comprising a plurality of microcapsules.
26. A composition as claimed in claim 25 wherein the composition comprises a plurality of microcapsules derived from a plurality of different types of fungal cells.
27. A composition as claimed in any one of the previous claims wherein the autolysed cell comprises a damaged cell membrane.

28. A composition as claimed in any one of claims 1 to 27 wherein the microcapsule substantially comprises a cell wall
29. A composition as claimed in any one of claims 1 to 27 wherein the microcapsule substantially consists of a cell wall.
30. A composition as claimed in any one of claims 1 to 27 wherein the microcapsule consists of a cell wall
31. A composition as claimed in any one of the previous claims wherein the composition further comprises water co – encapsulated with the encapsulatable material.
32. A composition as claimed in any one of the preceding claims, wherein the encapsulatable material comprises any one or more of a flavour, a fragrance, a pharmaceutically active compound, a phytoactive compound, a biocidally active compound, an antimicrobial or microbialstatic compound, a pesticidal compound, an insecticide, an avicide, an acaricide, a rodenticide, a molluscicide, a nematocide, a nutraceutical, an animal/bird/insect repellent compound, a cleaning agent, adhesive or adhesive component, a dye, an antioxidant, a skin-anti-wrinkle agent, an essential oil or a pheromone.

33. A composition as claimed in claim 32 wherein the encapsulatable material is lipophilic or comprises a lipophilic moiety.

34. A composition as claimed in any one of claims 32 or 33 wherein the encapsulatable material is lipid soluble.

35. A composition as claimed in any one of claims 32 to 34 wherein the encapsulatable material is derived from a lipophobic compound and which is made lipophilic by chemical modification.

36. A composition as claimed in claim 32 wherein the encapsulatable material is hydrophilic, hydrophobic or may comprise hydrophilic and hydrophobic moieties.

37. A composition as claimed in claim 36 wherein the encapsulatable material is water soluble.

38. A composition as claimed in any one of claims 32 to 37 wherein the encapsulatable material further comprises a carrier.

39. A composition as claimed in any one of claims 32 to 38 wherein the encapsulatable material is in liquid form or solution at standard temperature and pressure.

40. A composition as claimed in any one of claims 38 or 39 wherein the carrier comprises any one or more of the following:

- h) primary alcohols within the range C4 to C12;
- i) secondary and tertiary alcohols;
- j) glycols;
- k) esters, particularly esters having straight carbon chains greater than 2 and less than or equal to 12;
- l) aromatic hydrocarbons;
- m) any aromatic lipophilic oil with no straight chain branch greater than 12 Carbons; and
- n) carboxylic acids between C3 and C12

41. A composition as claimed in claim 40 wherein the carrier is organic and has a molecular weight in the range of 100 – 700.

42. A composition as claimed in claim 40 or 41 wherein the carrier comprises any one or more selected from the following: Alkanes, alkenes, alkynes, aldehydes, ketones, monocyclics, polycyclics, heterocyclics, monoterpenes, furans, pyroles, pyrazines, azoles, carboxylic acids, benzenes, alkyl halides, alcohols, ethers, epoxides, esters, fatty acids, essential oils.

43. A composition as claimed in claim 42 wherein the carrier comprises any one or more of the compounds selected from table 1.

44. A composition as claimed in any one or more of the preceding claims wherein the composition is formulated as a dry or liquid (emulsion or suspension) syrup, a sachet, a chewable, a chewing gum, an orodispersible, a dispersible effervescent, a dispersible tablet, a compressed buccal tablet, a compressed sublingual tablet, a chewable tablet, a melt-in-the-mouth, a lozenge, a paste, a powder, a gel, a tablet, a compressed sweet, a boiled sweet, a cream, a suppository, a snuff, a spray, an aerosol, a pessary, or an ointment.
45. A composition as claimed in any one or more of the preceding claims, wherein the encapsulatable has a positive partition coefficient ( $\text{LogP}_{ow}$ ) greater than 0.1, in the range 0.1-10, in the range 0.5 - 10, in the range 0.5-7.0, or in the range 2.0-7.0.
46. A composition as claimed in any one of the previous claims, wherein the encapsulatable material has a pH in the range pH1.0 –12.0 or pH4-9.
47. A composition as claimed in any one of the previous claims wherein the encapsulatable material is present in an amount from 1-50 g/100g of composition.
48. A method of producing a microbially encapsulated exogenous material, wherein the microcapsule comprises an autolysed microbial cell and the method

comprises the steps of contacting the autolysed microbial cell with the encapsulatable material such that the encapsulatable material is substantially encapsulated within the autolysed microbial cell.

49. A method of encapsulation comprising the steps of:

- c) admixing a microbial cell with an exogenous compound such that at least some of the exogenous compound is encapsulated; and
- d) admixing an autolysed microbial cell with the admixture to encapsulate any non-encapsulated exogenous material.

50. A composition as claimed in any one of claims 1 to 47 for use as a pharmaceutically active composition.

51. A composition as claimed in any one of claims 1 to 47 and 50 wherein the carbohydrate content of the autolysed cell is at least 30% carbohydrate by dry weight of the microcapsule.

52. A composition as claimed in claim 51 wherein the carbohydrate content of the autolysed cell is at least 50% carbohydrate by dry weight of the microcapsule.

53. A composition as claimed in claim 52 wherein the carbohydrate content of the autolysed cell is at least 75% carbohydrate by dry weight of the microcapsule.

54. A composition as claimed in claim 53 wherein the carbohydrate content of the autolysed cell is at least 80% carbohydrate by dry weight of the microcapsule.

55. A composition comprising a microcapsule and an encapsulatable material, wherein the microcapsule comprises a microbial cell having a cell wall and at least 30 % carbohydrate by dry weight, and wherein the encapsulatable material is substantially encapsulated within the cell wall.

56. A composition as claimed in claim 55 wherein the microbial cell is autolysed.



Fig. 1

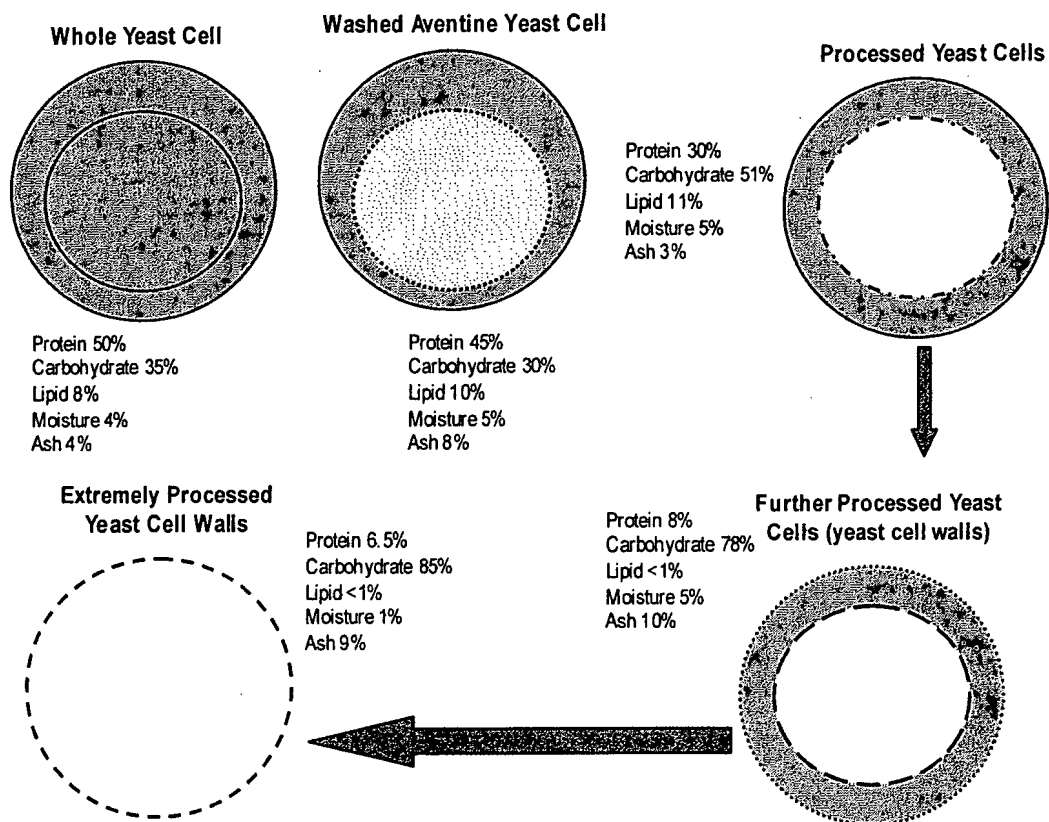
**Biochemical Differences Between Whole Yeast and Yeast Cell Walls**

Fig. 2

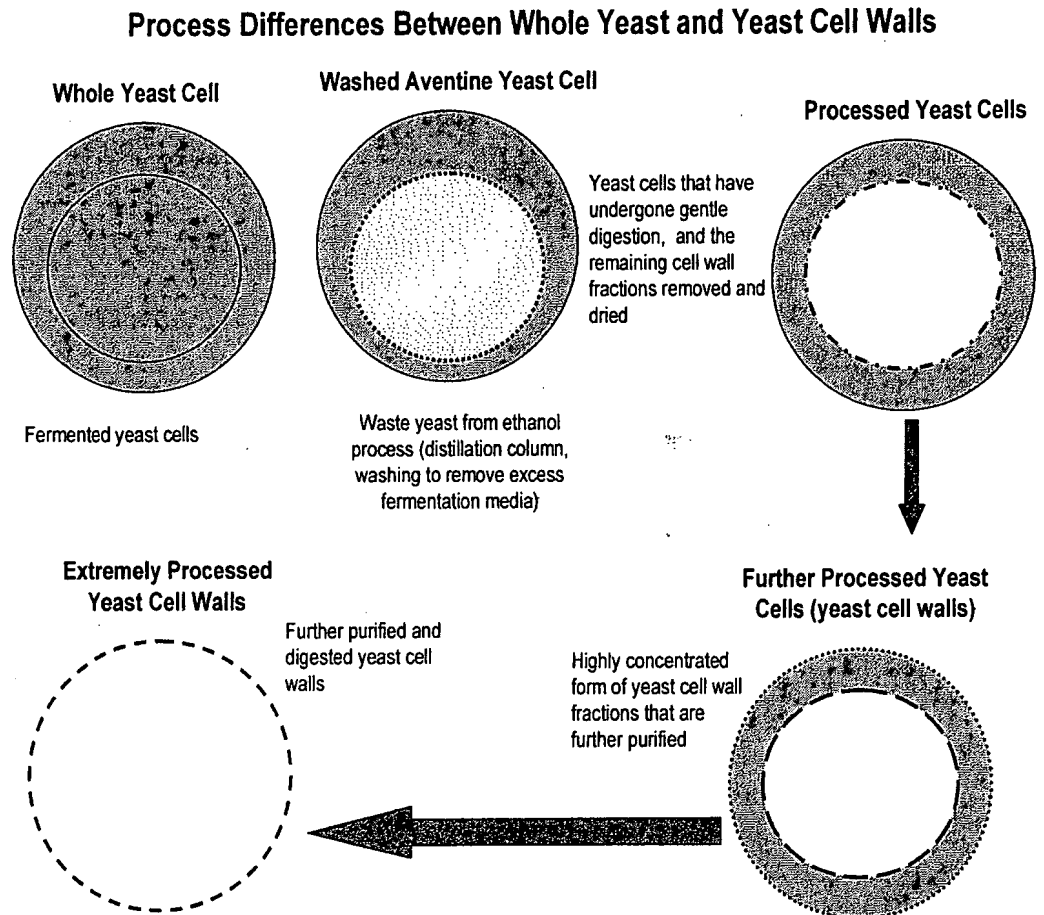
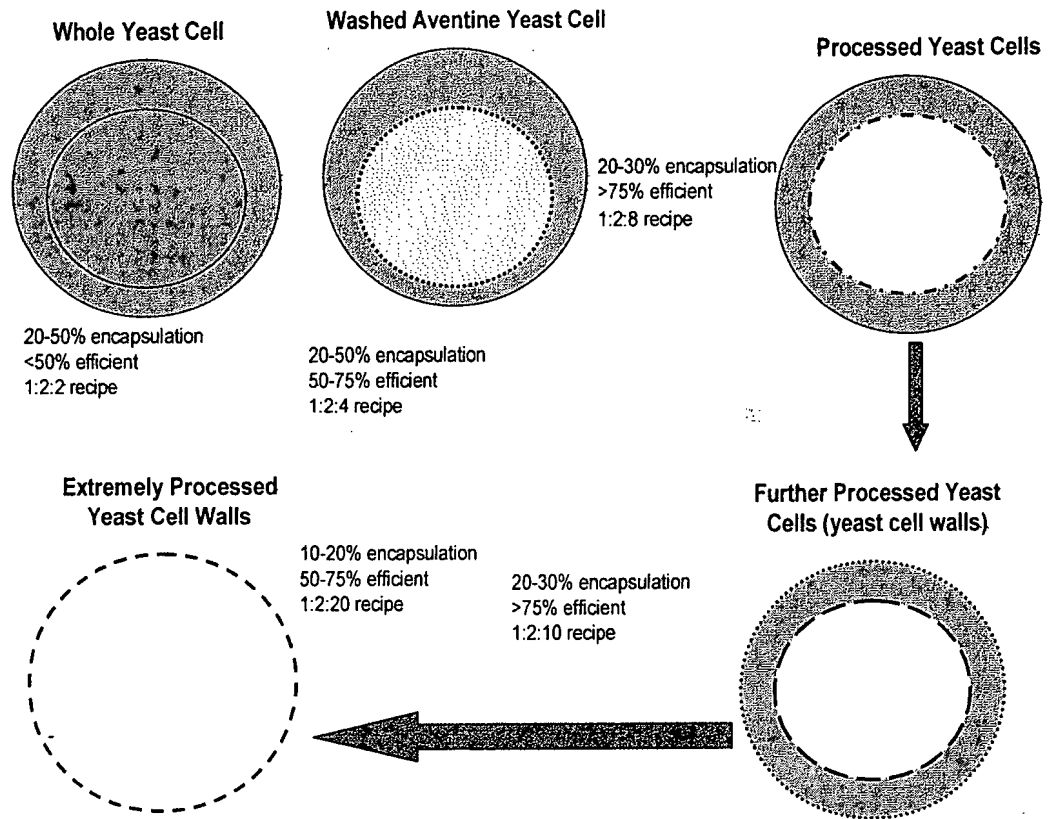


Fig. 3

**Recipe and Encapsulation Differences Between Whole Yeast and Yeast Cell Walls**

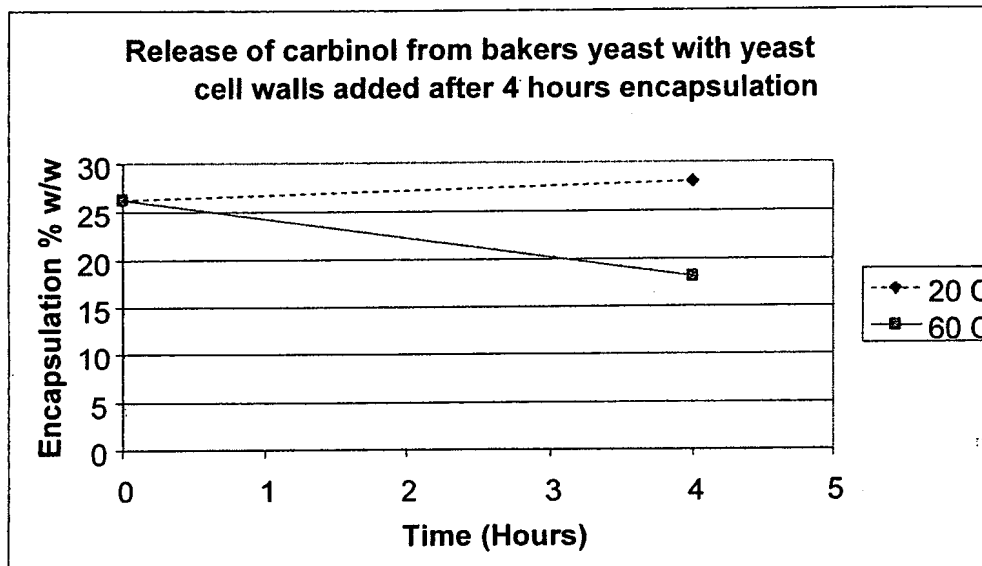


Fig. 4

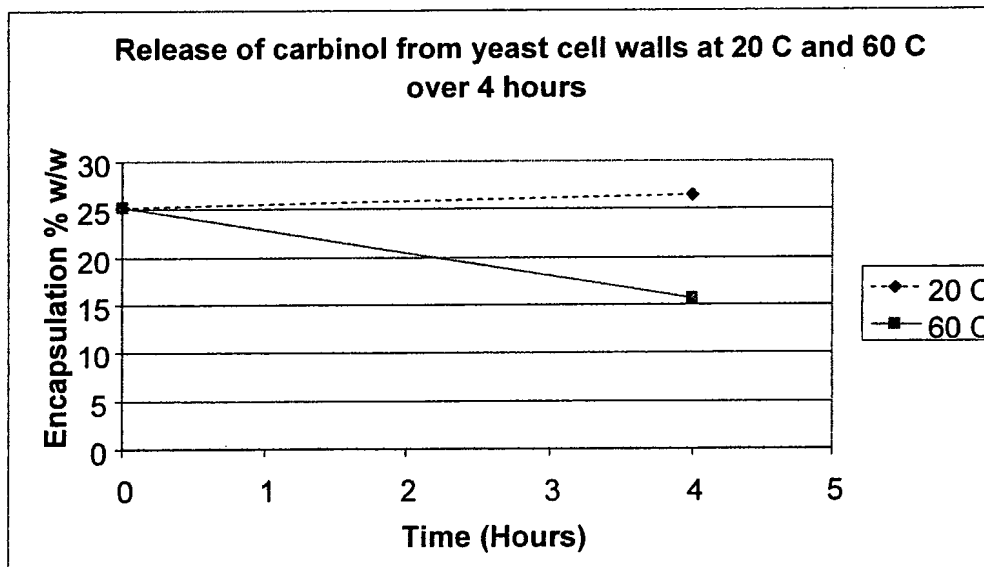


Fig. 5

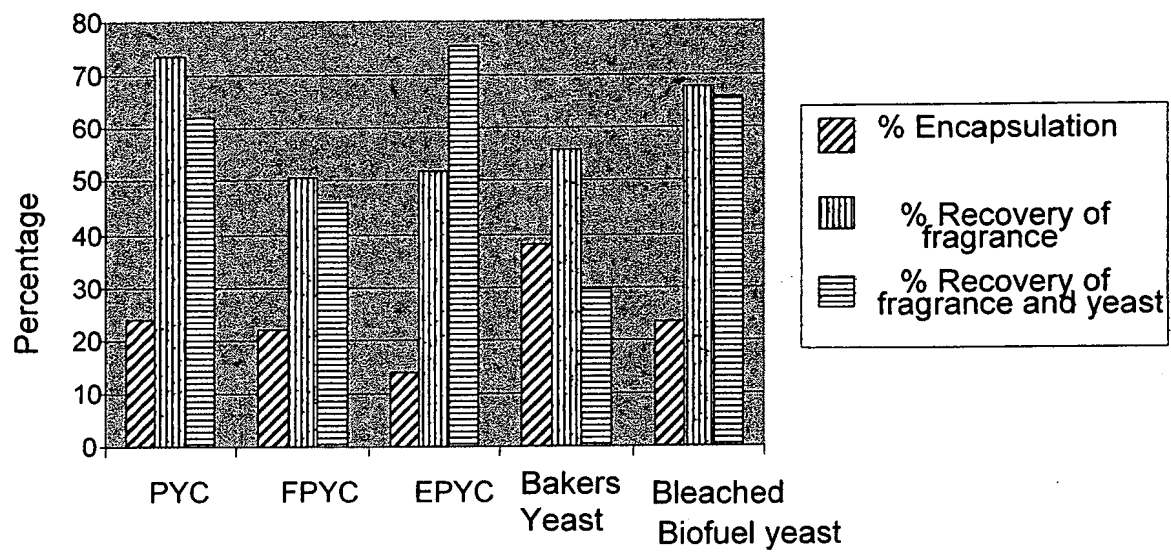


Fig. 6

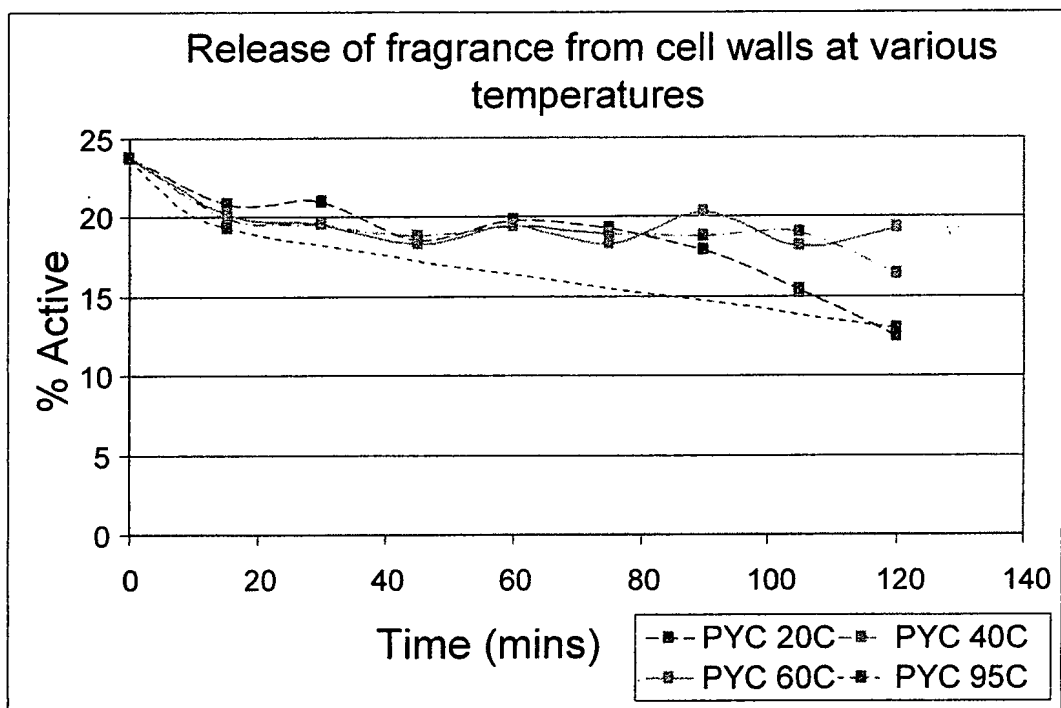


Fig. 7 a

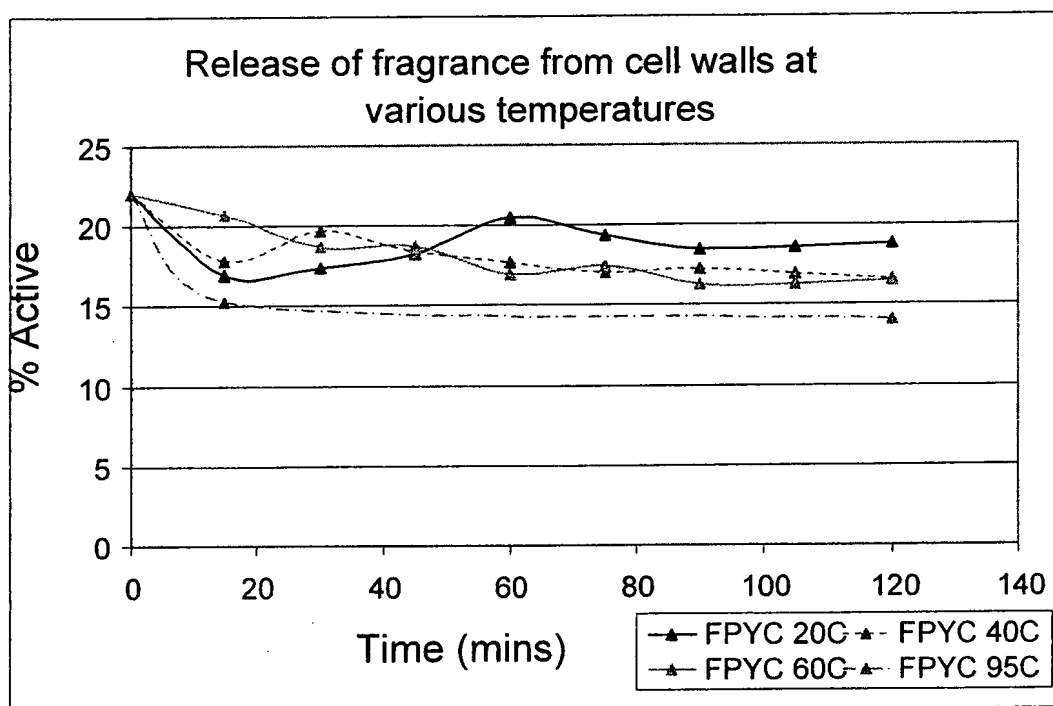
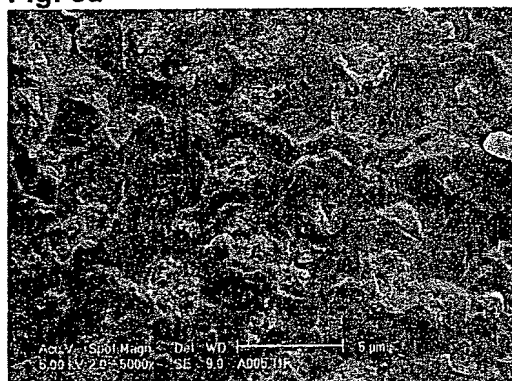


Fig. 7b

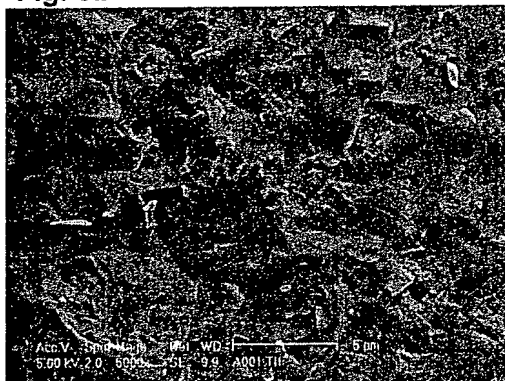
Fig. 8a



A-01

T-5

Fig. 8b



A-02

T-1

Fig. 8c



A-03

T-2

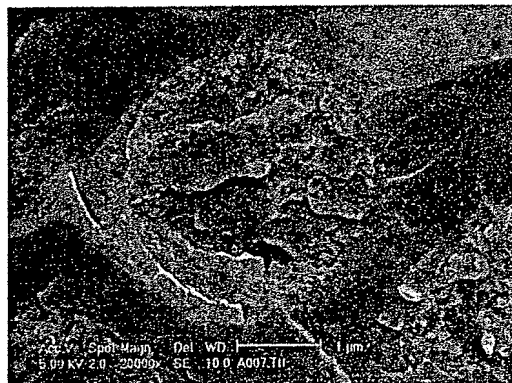
Fig. 8d



A-04

T-3

Fig. 8e



A-05

T-6

Fig. 8f

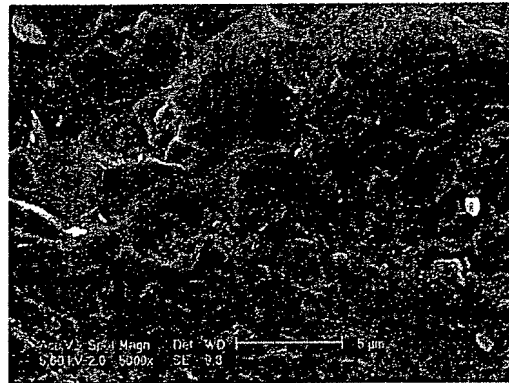


A-06

T-4



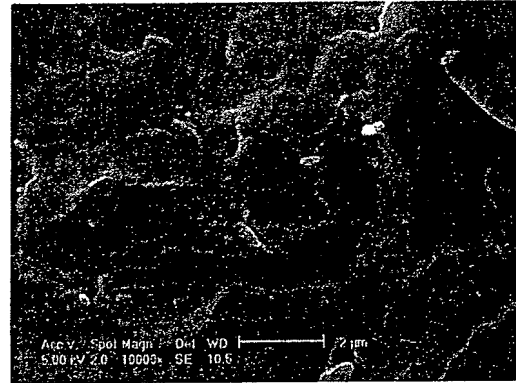
Fig. 9a



A-09

T-9

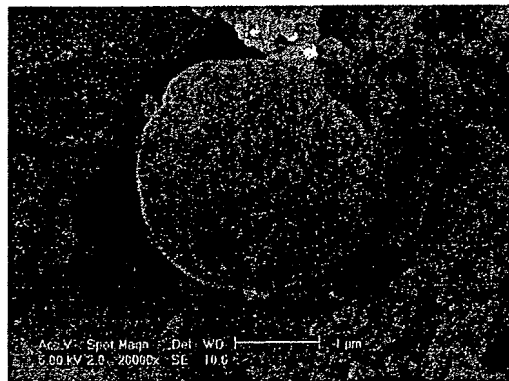
Fig. 9b



A-10

T-10

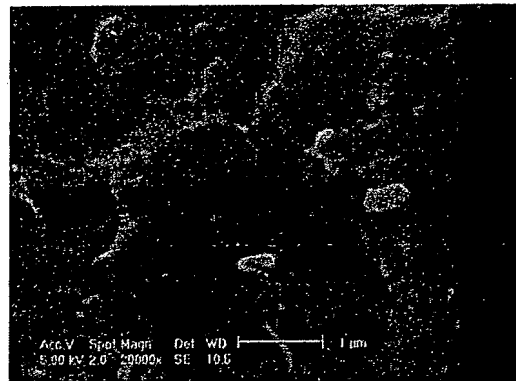
Fig. 9c



A-13

T-16

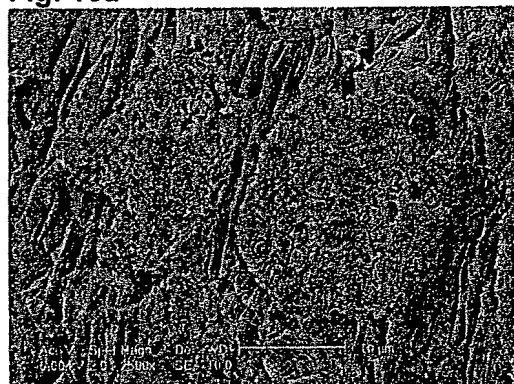
Fig. 9d



A-14

T-12

Fig. 10a



A-17

T-17

Fig. 10b



A-18

T-21

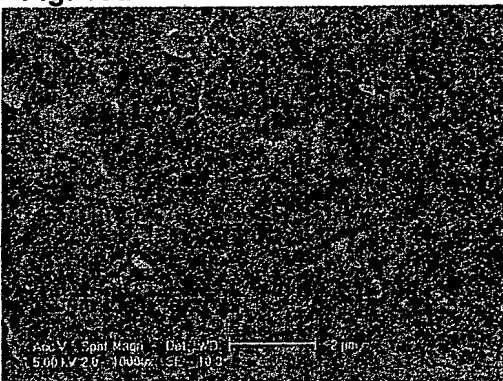
Fig. 10c



A-19

T-22

Fig. 10d



A-20

T-20

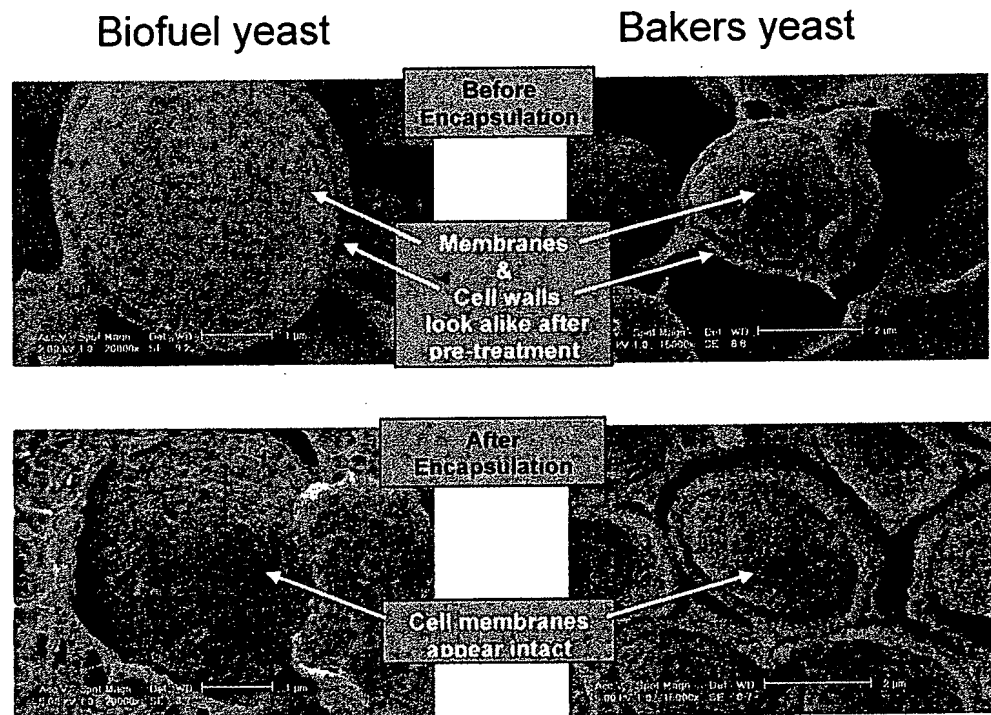
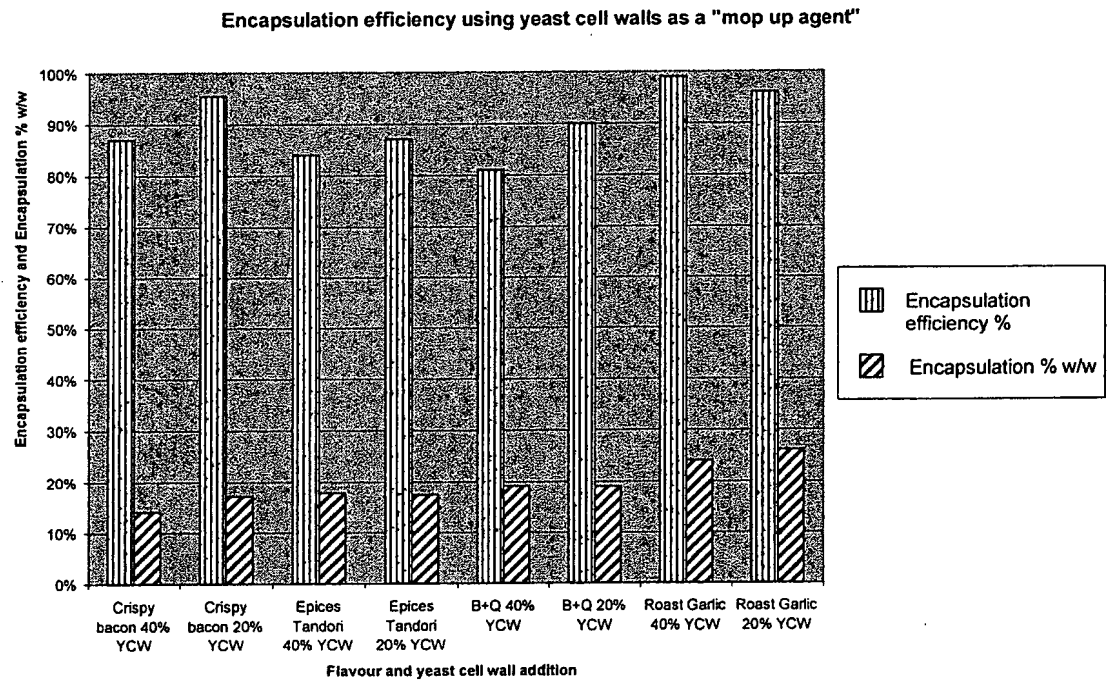


Fig. 11

**Fig. 12**